Objectives

- Try to answer some of the frequently asked questions about:
  - The cause of the PNH
  - The clinical presentation of PNH
  - Diagnosing PNH
  - The complications of PNH
  - New treatments for PNH

What is PNH?

- A disorder of blood affecting all the cells which come from the bone marrow.
- The disease is quite rare, only 10,000 patients in the US and Europe.
- There is no ethnic preference for the disorder.
- It may present early or late in life.
- The manifestations may be “classic” or obscure.

What is PNH?

- PNH is due to a mutation in a gene in a blood stem cell.
- The gene is called the PIG-A gene and is located on the X chromosome.
- In most cases of PNH, the change in the gene (mutation) is acquired, not something you are born with. When and why is unknown.
- The gene contains the genetic information for the GPI anchors which link proteins to the cell membrane.
What is PNH?

Evolution of PNH in Marrow

NORMAL CLONES

ABNORMAL CLONE

What is PNH?

Copies itself for a new cell

When the cell divides, the new cells then contain the new genetic information

What is PNH?

GPI-AP Biosynthesis: Involves 10 Steps and >20 Genes

Plasma Membrane

Endoplasmic Reticulum

What is PNH?

As a result of the PIG-A mutation, there is little of no GPI anchor produced.
- PNH II cells- mild reduction
- PNH III cells- severely reduced.
- When the anchor is reduced, certain proteins can’t attach to the cells.
- The most important proteins for PNH are CD 59, CD55.

What is PNH?

Many normal people have very small numbers (perhaps 6 per 1,000,000 bone marrow cells)

- In PNH, the abnormal cells have an advantage and become a major population in the marrow and blood (anywhere from 1% to over 90%)
  - This may be a result of change in the immune system— inability to recognize something foreign.
  - Or it may be related an immune attack on and resultant damage to the bone marrow, leading to poor production of blood from the marrow

PNH Clinical Features Aplastic Anemia

- Some PNH patients have aplastic anemia or a history of aplastic anemia
- Many PNH patients have evidence of a bone marrow that doesn’t work well or well enough to maintain normal blood counts
- Therefore, whatever causes aplastic anemia (immune suppression or dysregulation or damage to the stem cells) may allow PNH to develop
What is PNH?
Complement

- Complement is a group of blood proteins that act together to help the body get rid of pathogens such as microbiological invaders.
  - One of the ways it does this is by penetrating the membrane (outside surface) of the invading bacteria or viruses.
- Complement induces inflammation and recruits inflammatory white blood cells to the area of injury or pathology. This can help trap and digest a pathogen or damaged cells.
- When complement proteins bind to PNH blood cells, the cells are destroyed.

Complement circulates in an inactive form

- It is activated spontaneously and by a variety of events.
  - It is normally activated more at night.
  - It is more active with infections, trauma, vaccinations, surgery, immune complexes, autoimmune diseases.

Complement activity is regulated by proteins in the blood and on the membranes of the cell.

- GPI linked proteins on the cell surface interfere with complement to prevent breakdown (lysis) of the cell membrane.
  - The most important of these are CD59, and CD55 which is missing on the abnormal cells of PNH.
  - For this reason, PNH red cells are extremely sensitive to very small amounts of activated complement.

Absence of CD59 Allows Terminal Complement Complex Formation

Multimeric C9 Lesions on PNH Cell membranes

- Complement attacks the red cells and they break up (hemolysis).
  - This releases hemoglobin (the red pigment in red cells) into the plasma.
  - Causes anemia.
  - Pieces of the membrane come off.
- The white cells release granule contents and change to express other proteins.
- The platelets form vesicles (membrane blisters) and activate.
**What is PNH?**

Normal red blood cells are protected from complement attack by a shield of terminal complement inhibitors. Without this protective complement inhibitor shield, PNH red blood cells are destroyed.

**Complement activation**

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**Clinical Features**

- Some of the hemoglobin passes through the kidneys and into the urine, causing red to dark brown urine (hemoglobinuria)
  - This causes a loss of iron from the body
  - In the long run, this may damage the kidney
- Free hemoglobin binds nitric oxide causing vascular and smooth muscle spasm
- Causes inflammation

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**Clinical aspects**

- Vascular (arterial constriction, HBP)
- Pulmonary artery pressure increase (PHTN)
- Spasm of the esophagus
- Abdominal pain
- Erectile dysfunction
- Other symptoms such as "fatigue"
- Platelets are more "reactive"

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**Clinical Features**

- Hemolytic anemia due to complement activation
  - Hemoglobinuria and kidney damage
  - Anemia to a variable degree
  - Effects of NO depletion - HBP, smooth muscle dystonia, reduced blood flow to the kidney and lungs
- Impaired bone marrow function

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**Bone Marrow Failure Syndromes**

- AML
- AA
- MDS
- PNH
- MPD
- LGL
- PRCA

[Young NS. Ann Intern Med. 2002 Apr 2;136(7):534-46]
Normal Hematopoietic Stem Cells

Step 1
Somatic Mutation of PIG-A

Step 2
Immunological Attack
Selective Damage

Step 3
Growth Advantage

GPI-deficient Cell
Selected Cells
Expansion Cells

- Expansion of the clone size is necessary to result in clinical PNH
- The need for both selection and expansion may explain the rarity of PNH

What is PNH?
Clinical Features

- Thrombosis (Blood clots)
  - Often in unusual places (liver veins, abdominal veins, cerebral veins, dermal veins)
  - Can damage kidneys
- Fatigue – overwhelming, poor correlation to level of hemoglobin
- Inflammation
- Anemia
- Pulmonary Hypertension

What is PNH?
Diagnosis of PNH

- Historical test – Sucrose hemolysis, Hamm’s test no longer used
- Flow cytometry on peripheral blood is the gold standard for diagnosing PNH
- Both granulocytes and erythrocytes should be tested
  - Erythrocytes alone are not sufficient due to hemolysis and dilution effect of transfusions
- Multiple monoclonal antibodies against GPI-anchored proteins (such as CD59 or CD55) are used
- PNH blood cells (PNH clone) are cells that are missing GPI-anchored proteins

What is Soliris®?

- Monoclonal antibody (protein) that blocks complement at C5 preventing the formation of the terminal complement complex
- Quickly and markedly reduces hemolysis
  - Stops hemoglobinuria
  - Increases hemoglobin level
  - Reduces transfusions
  - Hemoglobin may not be quite normal

Management Options for PNH

Generally conservative, supportive, and dependent on symptom severity

- Transfusions
- Anticoagulants
- Supplements
  - Folic acid
  - Iron
  - Erythropoiesis stimulating agents
- Steroids/androgen hormones
- Allogeneic bone marrow transplant (limited eligibility)
- Complement inhibition

What is PNH?

What is Soliris®?
**SOLIRIS Blocks Terminal Complement**

- SOLIRIS binds with high affinity to C5
- Terminal complement activity is blocked
- Proximal functions of complement remain intact
  - Weak anaphylatoxin
  - Immune complex and apoptotic body clearance
  - Microbial opsonization

**Reduction in Chronic Complement-Mediated Hemolysis as Measured by LDH**

- TRIUMPH placebo patients switched to Soliris after Week 26
- All TRIUMPH patients entered the long-term extension study

**D.T., urine score 2 weeks before & after Eculizumab**

**Effect of Complement Inhibition on Ability to Maintain a Good Hemoglobin**

- Placebo
- Eculizumab

**Effect of Soliris® on Transfusion in PNH**

- Placebo
- Eculizumab

**What is the effect of Complement inhibition in PNH?**

- Stops the symptoms associated with intravascular hemolysis
  - "Fatigue"
  - Esophageal and abdominal spasm
  - Erectile dysfunction
  - Improves sense of well being
  - Reduced the need for transfusion
- Appears to reduce thrombosis (blood clots)
Effect of Eculizumab on the Thromboembolic Event Rate in PNH

N = 195 Patients on Antithrombotics

94% reduction in TE event rate with eculizumab

Pre-Eculizumab: 92% reduction in TE events with eculizumab


(\(P<.0000001\))

(\(P<.001\))

What is the Effect of Complement inhibition?

Improves kidney function
- reduced hemoglobinuria and iron deposition
- Reduced thrombosis

Improves hypertension
- May in part be due to availability of nitric oxide

Pregnancy in PNH

Pre-eculizumab:
- Increased fetal loss
- Increased risk of thrombosis*
- Increased transfusion requirements

Post Eculizumab*:
- Improved fetal outcomes
- No major fetal abnormalities
- Reduced maternal morbidity
- Reduced risk of thrombosis*

Anticoagulation with heparin or LMWH required throughout the pregnancy and for 6-12 weeks post partum

**Eculizumab category C

Side Effects of Eculizumab Treatment

- Susceptibility to sepsis by meningococcal organism
- All patients must be vaccinated at least 2 weeks before starting Soliris
- All patients must know to seek medical help at once when fever happens
- All patients must carry cards describing this complication
- Headache – first week or 2
- Cost
- Inconvenience
  - Must be given every 12-14 days by vein
**What Eculizumab Cannot Do**

- Does not appear to improve impaired bone marrow function
  - Low white count or low platelet count may persist in some patients, especially if it is due to aplastic anemia
  - Other treatments may be indicated
    - Bone marrow transplantation
    - ATG and other immunosuppressives

**When is Eculizumab Ineffective or Less Effective**

- Patient has been incorrectly diagnosed with PNH.
- C5 polymorphism
- Patient has a very small PNH clone (less than 10%)
  - bone marrow failure - AA
- Breakthrough- inadequate dosing vs increased complement activation
- Extravascular hemolysis

**Breakthrough Hemolysis with Eculizumab**

- Eculizumab 1/2, 10-12days
- Types of breakthrough - insufficient dose vs more rapid clearance vs increased complement activation
  - day 8 LDH within normal limits but LDH increases prior to next dose on day 15
  - CH 50 increases, measure a trough level trough (level decreases below 15 ug/ml) prior to day 15
- Recommended treatment:
  - increase frequency of the dose to q 12 days (per PI) or give extra dose
  - increase dose to 1200 mg q 14 days.
  - Experimental- SQ daily vs 1210

**Extravascular clearance**

- Accumulation of C3b/d/g on cells with clearance through the spleen.
- Complement Receptor (CR)1 modulates C3 deposition on RBC
- Genetic variants H/H, H/L, L/L
- polymorphism L/L(low expressor) increase C3 on membrane
  - 7 times more likely to require transfusions

Treatment: transfusions, prednisone (?), splenectomy(?)
Experimental- APL2

**Residual hemolysis in PNH: Breakthrough vs extravascular hemolysis**

- Extravascular hemolysis
  - Accumulation of C3b/d/g on cells with clearance through the spleen.
  - Complement Receptor (CR)1 modulates C3 deposition on RBC
  - Genetic variants H/H, H/L, L/L
  - polymorphism L/L(low expressor) increase C3 on membrane
    - 7 times more likely to require transfusions

Treatment: transfusions, prednisone (?), splenectomy(?)
Experimental- APL2

**Thank you**